Plant Responses to Water Deficit and Shade Stresses in Pigweed and Their Influence on Feeding and Oviposition by the Beet Armyworm (Lepidoptera: Noctuidae)

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ABSTRACT Water deficit and shade stress in weed-infested crops could alter plant growth and biochemistry and feeding and oviposition by the beet armyworm, *Spodoptera exigua* Hübner. Palmer amaranth pigweed, *Amaranthus palmeri* S. Wats., was grown under 25% of full watering (water deficit), 30% of full light (shade), or combined stress. All treatments decreased plant height and weight. Shade and combined stresses decreased leaf counts and increased leaf water content. Water deficit stress increased leaf water potential, soluble protein and carbohydrate contents, peroxidase activities, and accumulations of 10 individual free amino acids (FAAs), summed essential FAAs, and total FAAs. Combined stress increased water potential, soluble carbohydrates, 12 individual FAAs, summed essential FAAs, and total FAAs. Shade stress decreased water potential, soluble carbohydrates, seven individual FAAs, and essential FAAs. Beet armyworm larvae consumed similar leaf areas on water deficit–stressed and nonstressed plants and larger areas on plants grown under shade or combined stress. Larval survival was reduced, and time to pupation was higher on shade-stressed leaves. Adult females deposited more eggs on shade and combined stress plants and fewer eggs on water deficit-stressed plants compared with controls. Beet armyworm feeding and oviposition responded to variation in water content. Stress-induced changes in nutrients were not tied to insect preference but could have negatively influenced survival under shade stress. The results have implications for the plant stress hypothesis and for the use of pigweeds for beet armyworm detection.

KEY WORDS cotton pest, drought, plant stress, shading, weeds

PLANTS EXPERIENCE MULTIPLE ABIOTIC and biotic stresses in the field. Drought conditions can lead to water deficit stress, which influences plant growth and productivity (Boyer 1982, Saranga et al. 2001, Showler and Moran 2003), causes membrane damage, and stimulates molecular signal transduction and hormone activation (Knight and Knight 2001). Decreases in height, weight, and leaf area (Liu and Stutzel 2002, Yan et al. 2003), leaf wilting, and decreases in water potential (Kishor et al. 1995) are all symptomatic of water deficit stress. Soluble protein levels change (Chandra et al. 1998, Garg et al. 2001), and soluble carbohydrates increase (Pinheiro et al. 2001, Streeter et al. 2001). Increased peroxidase enzyme activity facilitates lignification and could ameliorate oxidative stress under water deficit (Smirnoff 1993). Protein breakdown and free amino acid (FAA) biosynthesis (Ingram and Bartels 1996, Bray 1997) lead to increases in specific FAAs such as proline and changes in broad FAA profiles (Showler et al. 1990, Streeter et al. 2001, Showler 2002).

Shade stress, one of many forms of stress caused by weed growth, can contribute to crop production losses (Showler and Reagan 1991, Showler and Greenberg 2003), decrease plant height, weight, and number of leaves (Rajcan et al. 2002), and influence FAA profiles (Showler 2002). Shaded tissues often have elevated nitrogen and water content and reduced carbon-based defenses such as phenolics (Crone and Jones 1999, Henriksson et al. 2003). Little is known about the effects of shade on metabolites such as peroxidases, soluble carbohydrates, and FAAs in crop weeds.

Water, protein, carbohydrates, free essential amino acids, and balances among these components are all important to insect feeding and survival (Mattson and Haack 1991, Nation 2002, Chapman 2003). Water deficit and shade stresses can alter plant nutritional balances, but effects on insects are variable. Changes in protein and FAA content in water deficit–stressed plants can favor insect feeding, growth, or reproduction (White 1984, Mattson and Haack 1991), make plants less suitable for herbivores (English-Loeb et al. 1997, Showler and Moran 2003), or have no effects (Waring and Cobb 1992). Shade stress increases feeding by leaf beetles, *Plagiodera versicolora* Laich, on

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cottonwood, *Populus deltoides* L. Bartr. (Crone and Jones 1999), and larval survival of the autumnal moth, *Epirrita autumnata* Bhk., on birch, *Betula pubescens* ssp. *czerepanovii* (Orlova) Hämet-Ahti (Henriksson et al. 2003).

Smooth pigweed, *Amaranthus hybridus* L., is preferred for oviposition and larval feeding over cotton, *Gossypium hirsutum* L., by the beet armyworm, *Spodoptera exigua* Hübner, in part because of pigweed's superior accumulations of amino acids (Showler 2001) that are essential for growth and development (Nation 2002). Surveys of pigweed could be used to predict movement of beet armyworms into cotton (Sappington et al. 2001, Carroll et al. 2003). Water deficit increases beet armyworm feeding and oviposition in cotton but reduces pupal weight (Showler and Moran 2003). The purpose of this study was to assess the effects of water deficit and shade stress on plant phenology and selected nutritional components in Palmer amaranth pigweed, *Amaranthus palmeri* S. Wats., and on beet armyworm feeding, survival, and oviposition.

Materials and Methods

Plants and Insects. Palmer amaranth pigweed seeds were propagated from greenhouse-grown plants at the USDA-ARS Kika de la Garza Subtropical Agricultural Research Center (KSARC), Weslaco, TX. Seeds were planted in Sunshine Mix No. 1 soil (Sungro Horticulture, Bellevue, WA) in 5.5-liter pots with 4-cm deep trays underneath to retain excess water, in a greenhouse maintained at 25–32°C under ambient light. Plant age was set to zero when cotyledons were observed. Seedlings were thinned to two per pot when they were 4 d old. Peters Professional 17–17–17 N-P-K fertilizer (Scotts-Sierra Horticultural Products, Marysville, OH) was applied (15.8 g/liter solution, 100 ml per pot) when seedlings were 1 wk old. All plants were irrigated every 2 d to soil saturation until the assays commenced, and 3-wk-old plants were tied to wooden stakes. Second- and third-instar beet armyworms were obtained from a colony started from wild moths collected in the Lower Rio Grande Valley of Texas in 1997 and were reared on a soybean-wheat germ diet (Shaver and Raulston 1971) in environmental chambers at 30°C, 85% RH, with a 13:11 L:D photoperiod.

Water Deficit and Shade Stress Treatments. Pots containing 3-wk-old seedlings were randomly assigned to treatments of 25% of full watering (water deficit stress), 30% of full light (shade stress), 25% of water and 30% of light (combined stress), or full light and watering (control), with separate controls for each stress treatment (n = 20 pots per treatment). Shade and combined stress pots were placed under 70% shade cloths (illuminance under sunlight in greenhouse, May 2004, 995 μmol/m^2 per second; under shade cloth, 382 μmol/m^2 per second). Every 4 d, water deficit and combined stress pots received 500 ml water, and shade stress and control pots received 2,000 ml. Pots were rearranged weekly. After 2 wk, phenological measurements and leaf samples were taken, and beet armyworm feeding, oviposition, and survival assays began.

Plant Phenology. The shoot height of the taller plant in each pot was measured at the start and end of the 2-wk treatment period. The internode distance between the two lowest nonsenescent leaves on this plant was also measured at the end (n = 10 or 20 plants per treatment). The water potential of one fully expanded leaf (four to five nodes from the shoot apex) was measured using balance pressures detected with a model 610 pressure bomb (PMS Instrument Co., Corvallis, OR), and the number of leaves and fresh weight of all live, above-ground plant parts were determined (n = 10 plants per treatment). All fully expanded green leaves on the main shoot and lateral shoots were excised, frozen on dry ice, and stored at −80°C for plant biochemical analyses (n = 14–15 plants per treatment for water deficit stress; n = 10 plants per treatment for shade and combined stress). Water content was determined by weighing frozen ground leaf samples before and after 72 h of lyophilization at −40°C.

Plant Biochemical Analyses. Soluble protein and peroxidases were extracted from fresh 0.3-g samples by homogenization in 0.01 M sodium phosphate buffer (10 ml/g sample, pH = 7) containing 0.75 mM ethylenediaminetetraacetic acid and 1% (wt:vol) polyvinylpyrrolidone. Extracts were centrifuged at 11,000 rpm for 15 min. To determine protein content, 6 μl supernatant was mixed with 200 μl Brilliant Blue G reagent (Sigma-Aldrich, St. Louis, MO), incubated for 5 min at 25°C, and read in a Benchmark microplate reader at 595 nm (Bio-Rad, Hercules, CA). Bovine serum albumin was used as a standard. Peroxidase activity was measured in a GeneSys-2 spectrophotometer (Spectronic, Rochester, NY) at 470 nm, 25°C, in a 1.5-ml reaction mixture (150 μl extract and 1,350 μl 0.025 M phosphate buffer, pH = 7, containing 0.25% [vol:vol] guaiacol substrate and 0.375% [vol:vol] hydrogen peroxide). Activity was expressed as the increase in absorbance per gram fresh weight per minute.

Total available carbohydrates (TACs) were extracted from 30 mg of lyophilized tissue with 1 ml deionized water stirred for 30 min at 25°C, incubated at 4°C for 16 h, and centrifuged at 13,000 rpm for 15 min. Fifty microliters of extract was mixed with 1,500 μl anthrone-sulfuric acid reagent (12.7 M H_2SO_4 in water containing 0.1% [wt:vol] anthrone and 0.1% [wt:vol] thiourea) and incubated at 60°C for 20 min, 0°C for 3 min, and 25°C for 20 min. Reactions were quantified at 625 nm. Glucose was used as a standard to calculate TAC content in milligrams per gram dry weight. A linear regression of dry weight on fresh weight of samples from nonstressed plants [fresh weight = dry weight(5.6214) + 0.1704; R^2 = 0.79] was used to convert TAC values to milligrams per gram fresh weight.

For FAA analysis, frozen leaf samples were homogenized in 0.1 N HCl (20 ml/g sample) with a Virtishear homogenizer (Virtis, Gardiner, NY). Seven milliliters of homogenate was centrifuged at 10,000 rpm for

30 min, and supernatants were stored at −80°C. FAAs were analyzed using reversed-phase high performance liquid chromatography (HPLC) with an Agilent 1100 Series (Agilent Technologies, Atlanta, GA) chromatograph using the method of Showler (2002). Flow rates were adjusted to 100 ml/min for solvent A (500 ml HPLC-grade water containing 0.272% [wt:vol] sodium acetate trihydrate, 90 μl triethylamine [TEA], and acetic acid to pH 7.2) and 1 ml/min for solvent B (100 ml HPLC water containing 1.36% sodium acetate trihydrate, acetic acid to pH 7.2, and 200 ml each of acetonitrile and methanol). A Sorbax Eclipse Triple A C_{18} fluorescence column (3.5 μm diameter, 150 cm; Agilent) was used, and absorbance was monitored at 262 and 338 nm for 48 min per sample.

Dual-Choice Feeding Assay. Water deficit, shade, or combined stress plants were paired with control plants based on shoot height (n = 7–10 pairs per comparison). Four fully expanded leaves, three to six nodes from the shoot tip, were excised from each plant, and areas were measured with a Li-Cor 3600 leaf area meter (Li-Cor, Lincoln, NE). Two leaves per treatment were arranged in alternating quarters of 12-cm-diameter petri dishes lined with moistened filter paper. Two dishes were used per plant pair. Four third-instar beet armyworm larvae were placed in the center of each dish and incubated for 24 h in a growth chamber at 25°C, 12:12 L:D photoperiod. Remaining leaf areas were measured and subtracted from initial areas, and areas consumed were summed across all leaves of each treatment in each plant pair.

No-Choice Feeding Assay. Thirty second-instar larvae were placed on the stem of the tallest plant in pots not used for biochemical sampling (n = 10 pots per treatment). Plants were caged using 0.8-mm mosquito netting. Larvae were removed after 72 h. Holes made by feeding were traced onto plastic transparency sheets. Nondamaged leaf parts and tracings were measured on the leaf area meter to determine total leaf area and area consumed by larvae.

Oviposition Assay. One pot with two control plants and one with two stressed plants were placed in a 1 by 1 by 0.5-m cage (n = 10 plant pairs per assay). Two newly mated, gravid females (Showler 2001) were released into the cage and provided with 5% (wt:vol) sucrose in water on cotton wicks immersed in 20-ml cups. After 24 h, plants were examined for egg masses. Numbers of eggs deposited on leaves were counted.

Survival Assay. Groups of 60 neonate larvae were confined with netting as above on pots containing two pigweed plants (n = 5 pots per treatment). Live larvae were counted after 10 d. In separate tests, detached leaves were fed to groups of four neonate beet armyworm larvae in 15-cm-diameter petri dishes lined with moist filter paper (n = 5 dishes per treatment). Leaves were changed every 24–48 h, depending on food depletion. After 10 d, surviving larvae were counted and placed without food into 500-ml cardboard containers filled with moistened potting soil. The average time to pupation, pupal weight, and length were determined for each group.

Statistical Analyses. To meet normality and homogeneity of variance assumptions, proportional FAA and larval survival data were arcsine-square root transformed, and peroxidase and TAC data were $\log(x + 1)$-transformed. The effects of water deficit, shade, and combined stresses on all phenological variables, protein and TAC content, peroxidase activity, larval survival, and pupal measures were analyzed with one-way analysis of variance (ANOVA) and Tukey mean separation ($P \leq 0.05$; SAS Institute 1999). Effects on final shoot height were examined with analysis of covariance (ANCOVA), using initial height as a covariate and omitting a nonsignificant treatment × initial height interaction. MANOVA and Pillai's trace were used to detect treatment effects on all FAAs. ANOVAs were performed on accumulations of individual FAAs, summed essential FAAs identified using Nation (2002), and total FAA accumulations. ANOVA was used to examine proportions consisting of five individual FAAs that comprised 10% or more of the total accumulation, and summed essential FAA content. Leaf areas consumed in dual-choice and no-choice feeding assays were analyzed with Pearson χ^2 and ANOVA, respectively. Oviposition data were examined with Yates' corrected χ^2 (Analytical Software 1998).

Results

Plant Phenology. All pigweed plants produced terminal inflorescences by the end of the 2-wk treatment period. Final shoot height was reduced 31% by water deficit, 26% by shade, and 45% by combined stress, and daily increases in shoot height were reduced 37, 31, and 52% by the three stress treatments, respectively (Table 1). Shoot fresh weights were reduced 35% by water deficit, 24% by shade, and 40% by combined stress (Table 1). The internode between the two lowest nonsenescent leaves was 1.4- and 1.5-fold longer in plants grown under shade and combined stresses, respectively, than in their respective controls, and these plants had 42 and 33% fewer leaves (Table 1). Leaf balance pressures (used to estimate water potential) were 1.1- and 1.4-fold greater in the water deficit and combined stress treatments than in controls and were reduced 35% by shade stress (Table 1). The water content of leaves was 8.4 and 7.8% greater in plants grown under shade and combined stresses, respectively, than in controls (Table 1).

Biochemical Measures. Soluble leaf protein content was 2.4-fold higher in water deficit–stressed plants than in controls (Table 2). TAC content was elevated 2.4-fold by water deficit and 1.9-fold by combined stress and was reduced 35% by shade stress (Table 2). Soluble peroxidase activity increased 5.7-fold under water deficit stress and 9.5-fold under shade stress (Table 2).

Pigweed leaf extracts contained up to 15 detectable FAAs (Table 3), including at least six essential FAAs (arginine, histidine, isoleucine, leucine, phenylala-

Table 1. Final plant height, change in height per day, fresh weight, internode length, no. leaves, water potential, and percent water content (mean ± SE) of pigweed plants grown under water deficit, shade, or combined stresses in comparison to nonstressed plants

Measurement	Stress type[a]			
	Treatment[b]	Water deficit	Shade	Combined
Final ht (cm)	Control	126.1 ± 5.1a	123.2 ± 4.2a	93.5 ± 1.3a
	Stress	87.6 ± 4.3b	91.7 ± 3.1b	51.6 ± 2.9b
	F, P	18.8, <0.001	26.3, <0.001	82.5, <0.001
Change in ht (cm/d)	Control	6.41 ± 0.29a	6.37 ± 0.24a	4.93 ± 0.10a
	Stress	4.04 ± 0.28b	4.40 ± 0.18b	2.35 ± 0.18b
	F, P	34.6, <0.001	41.8, <0.001	152, <0.001
Fresh wt (g)	Control	63.9 ± 4.4a	57.0 ± 3.0a	47.7 ± 3.6a
	Stress	41.8 ± 2.9b	43.3 ± 3.6b	28.4 ± 8.2b
	F, P	17.8, <0.001	8.67, 0.006	21.7, <0.001
Internode length (cm)	Control	4.61 ± 0.30a	7.22 ± 0.62b	5.03 ± 0.66b
	Stress	4.48 ± 0.43a	10.1 ± 0.57a	7.43 ± 0.56a
	F, P	0.06, 0.800	11.7, 0.002	7.71, 0.012
No. leaves	Control	60.1 ± 4.9a	62.0 ± 4.8a	92.3 ± 7.6a
	Stress	57.5 ± 3.2a	35.7 ± 2.8b	62.2 ± 8.2b
	F, P	0.19, 0.667	23.1, <0.001	7.27, 0.015
Water potential (barr)	Control	7.85 ± 0.26b	5.25 ± 0.17a	7.00 ± 0.32b
	Stress	9.00 ± 0.28a	3.40 ± 0.22b	9.90 ± 0.32a
	F, P	9.14, 0.007	43.9, <0.001	30.4, <0.001
Water content (%)	Control	80.7 ± 0.01a	83.0 ± 0.01b	81.0 ± 0.01b
	Stress	80.5 ± 0.01a	91.3 ± 0.00a	88.8 ± 0.01a
	F, P	0.04, 0.839	149, <0.001	56.6, <0.001

Means for each variable in the same column followed by the same letter are not significantly different (Tukey tests, $P > 0.05$).
[a] Water deficit, 25% of full watering and full natural light; shade, full watering and 30% of full light; combined, 25% watering and 30% light.
[b] *F* and *P* values from ANOVA; $n = 20$ in water deficit and shade treatments for final ht, change in ht, internode length, and fresh wt; df = 1,38 (df = 2,37 for ANCOVA on final ht); $n = 10$ in the combined stress treatment for final ht, change in ht and internode length, and for all stresses for no. leaves, water potential, and water content, df = 1,18 (df = 2,17 for ANCOVA on final ht).

nine, and valine). Two essential FAAs, lysine and threonine, and nonessential alanine were detected only in experiments involving shade and combined stress. Multivariate ANOVA analyses indicated significant effects of water deficit (Pillai's trace = 0.903; $F = 12.5$; df = 12,16; $P < 0.001$), shade (Pillai's trace = 0.986; $F = 18.4$; df = 15,4; $P = 0.006$), and combined stress (Pillai's trace = 0.991; $F = 27.9$, df = 15,4; $P = 0.003$) on FAA accumulation. Five FAAs comprised a 10% or higher proportion of the total FAA accumulation (Fig. 1). The responses of glutamate and aspartate were broadly similar across all stress treatments. Glutamate accumulations increased 2.8-fold under water deficit, 1.2-fold under shade, and 1.7-fold under combined stress (Table 3), and shade increased the proportion of total FAA accumulation consisting of glutamate by 7% ($F = 33.9$, df = 1,18, $P < 0.001$; Fig. 1). Aspartate accumulations were reduced 41 and 53% by shade and combined stress treatments, respectively (Table 3), and proportional aspartate declined by 15% under water deficit ($F = 64.6$, df = 1,27, $P < 0.001$), 7% under shade ($F = 20.7$, df = 1,18, $P < 0.001$), and 21% under combined stresses ($F = 180$, df = 1,18, $P < 0.001$; Fig. 1).

Besides glutamate and aspartate, the responses of nine other individual FAAs to water deficit and combined stresses were similar. Free arginine accumulations were elevated 1.5-fold and >400-fold, histidine 2.4- and 3.0-fold, isoleucine 4.1- and 5.3-fold, leucine 2.7- and 7.1-fold, proline 3.6- and 2.3-fold, and serine 3.7- and 1.6-fold by water deficit and combined stresses, respectively (Table 3). Glycine, phenylalanine, and valine accumulations showed the same trend, increasing nonsignificantly, 1.6-, 17.8-, and 3.9-fold, respectively, under combined stress treatment,

Table 2. Protein and TAC content and peroxidase activity (mean ± SE) in pigweed plants grown under water deficit, shade, or combined stresses in comparison to nonstressed plants

Variable	Stress type[a]			
	Treatment[b]	Water deficit	Shade	Combined
Protein content (mg/g FW)	Control	6.1 ± 0.4b	5.5 ± 0.5a	11.1 ± 0.4a
	Stress	14.7 ± 3.2a	5.8 ± 0.3a	11.2 ± 0.4a
	F, P	55.0, <0.001	0.43, 0.519	0.04, 0.836
TAC content (mg/g FW)	Control	30.0 ± 1.7b	22.0 ± 0.5a	28.9 ± 1.0b
	Stress	72.1 ± 12a	14.2 ± 0.7b	56.2 ± 3.6a
	F, P	17.9, <0.001	72.5, <0.001	79.1, <0.001
Peroxidase activity (ΔAbs_{470}/g FW)	Control	2.5 ± 0.6b	0.1 ± 0.1b	16.0 ± 1.8a
	Stress	14.1 ± 3.2a	0.9 ± 0.2a	14.5 ± 2.7a
	F, P	19.2, <0.001	16.0, <0.001	0.63, 0.437

Means for each variable in the same column followed by the same letter are not significantly different (Tukey tests, $P > 0.05$).
[a] Water deficit, 25% of full watering and full natural light; shade, full watering and 30% of full light; combined, 25% watering and 30% light.
[b] *F* and *P* values from ANOVA; $n = 14$–15 plants, df = 1, 27 (water deficit stress); $n = 10$ plants, df = 1,18 (shade and combined stresses).

Table 3. FAA content (pmol/μl; mean ± SE) of pigweed plants grown under water deficit, shade, or combined stresses in comparison to nonstressed plants

Amino acid[b]	Stress type[a]			
	Treatment[c]	Water deficit	Shade	Combined
Alanine	Control	ND	579.7 ± 37.0a	312.6 ± 28.8b
	Stress	ND	693.0 ± 55.5a	562.3 ± 43.0a
	F, P		2.89, 0.106	23.2, <0.001
Arginine	Control	36.0 ± 5.90b	4.6 ± 2.37a	0 ± 0b
	Stress	54.6 ± 5.20a	15.1 ± 6.40a	411.1 ± 142a
	F, P	5.54, 0.026	2.35, 0.142	8.29, 0.010
Aspartate	Control	428.8 ± 16.8a	455.1 ± 42.8a	490.9 ± 58.7a
	Stress	518.0 ± 75.9a	270.8 ± 20.8b	230.4 ± 29.0b
	F, P	1.40, 0.247	15.0, 0.001	15.8, <0.001
Glutamate	Control	471.6 ± 64.7b	702.7 ± 40.1b	556.6 ± 47.2b
	Stress	1,301.6 ± 125a	815.0 ± 35.2a	945.6 ± 66.8a
	F, P	36.3, <0.001	4.43, 0.050	22.6, <0.001
Glycine	Control	0 ± 0b	56.6 ± 3.96a	33.8 ± 2.95a
	Stress	28.1 ± 4.04a	49.3 ± 3.04a	47.7 ± 7.18a
	F, P	52.2, <0.001	2.13, 0.162	3.23, 0.089
Histidine	Control	17.8 ± 3.50b	28.3 ± 3.82a	20.4 ± 2.63b
	Stress	42.9 ± 8.38a	19.3 ± 2.88a	61.9 ± 7.91a
	F, P	8.05, 0.009	3.55, 0.076	24.8, <0.001
Isoleucine	Control	26.9 ± 5.34b	44.3 ± 6.39a	10.3 ± 3.23b
	Stress	110.6 ± 19.9a	29.3 ± 2.87b	54.6 ± 17.5a
	F, P	17.5, <0.001	4.57, 0.047	6.16, 0.023
Leucine	Control	36.1 ± 2.45b	47.5 ± 5.11a	8.0 ± 4.3b
	Stress	97.1 ± 23.1a	24.3 ± 2.47b	57.2 ± 20.7a
	F, P	7.37, 0.011	16.8, <0.001	5.42, 0.032
Lysine	Control	ND	5.8 ± 3.04a	0 ± 0a
	Stress	ND	0 ± 0a	3.6 ± 2.45a
	F, P		3.59, 0.074	2.12, 0.163
Phenylalanine	Control	26.6 ± 3.47b	22.7 ± 1.77a	1.9 ± 1.89a
	Stress	104.1 ± 21.1a	21.5 ± 1.64a	33.4 ± 16.0a
	F, P	14.0, <0.001	0.24, 0.631	3.85, 0.065
Proline	Control	163.2 ± 36.1b	114.1 ± 4.99a	63.4 ± 6.97b
	Stress	582.1 ± 124a	98.9 ± 27.3a	143.6 ± 36.2a
	F, P	11.2, 0.002	0.30, 0.591	4.73, 0.043
Serine	Control	156.1 ± 11.9b	128.1 ± 12.1a	96.3 ± 10.1b
	Stress	577.8 ± 104a	86.2 ± 8.36b	158.1 ± 16.3a
	F, P	17.3, <0.001	8.15, 0.011	10.4, 0.005
Threonine	Control	36.3 ± 1.03a	51.3 ± 4.85a	33.9 ± 4.27b
	Stress	37.2 ± 1.20a	25.7 ± 2.64b	53.4 ± 6.28a
	F, P	0.29, 0.592	21.6, <0.001	6.56, 0.020
Tyrosine	Control	ND	24.8 ± 4.85a	13.0 ± 3.06a
	Stress	ND	0 ± 0b	9.6 ± 3.72a
	F, P		123, <0.001	0.490, 0.493
Valine	Control	21.7 ± 3.28b	27.1 ± 3.27a	17.0 ± 6.18a
	Stress	67.3 ± 16.6a	11.2 ± 1.34b	67.0 ± 23.9a
	F, P	7.76, 0.010	20.1, <0.001	4.11, 0.058
Free essential amino acids	Control	201.4 ± 7.20b	233.2 ± 19.9a	94.8 ± 17.4b
	Stress	513.8 ± 78.4a	146.4 ± 15.0b	751.5 ± 221a
	F, P	16.9, <0.001	12.1, 0.003	8.82, 0.008
Total FAAs	Control	1,421.1 ± 70.6b	2,294.4 ± 114a	1,661.4 ± 145b
	Stress	3,521.5 ± 354a	2,159.6 ± 113a	2,949.0 ± 332a
	F, P	36.1, <0.001	0.70, 0.413	11.3, 0.004

Means in the same column for each FAA followed by the same letter are not significantly different (Tukey HSD, $P > 0.05$).

[a] Water deficit, 25% of full watering and full natural light; shade, full watering and 30% of full light; combined, 25% watering and 30% light; ND, not determined.

[b] Essential FAAs are in bold.

[c] *F* and *P* values from ANOVA; $n = 14$–15 plants, df = 1,27 (water deficit stress); $n = 10$ plants, df = 1,18 (shade and combined stresses).

and significantly, 28-, 3.9-, and 3.1-fold, respectively, under water deficit (Table 3). Under combined stress treatment only, alanine increased 1.8-fold and threonine increased 1.6-fold. Water deficit increased the proportion of total FAAs consisting of serine by 5% ($F = 7.78$, df = 1,27, $P = 0.010$) and combined stress increased arginine proportions by 12% ($F = 40.0$, df = 1,18, $P < 0.001$; Fig. 1). In contrast to water deficit and combined stresses, shade stress had no effect on free arginine, glycine, histidine, phenylalanine, or proline accumulations and decreased isoleucine by 34%, leucine by 49%, serine by 33%, threonine by 50%, and valine by 59% (Table 3). Shade stress also decreased tyrosine to nondetectable levels (Table 3) and decreased proportional serine by 1.6% ($F = 11.4$, df = 1,18, $P = 0.003$; Fig. 1).

Summed essential FAA accumulations were elevated 2.9- and 7.9-fold by water deficit and combined stresses, respectively, and total FAA accumulations increased 2.5- and 1.7-fold, respectively (Table 3).

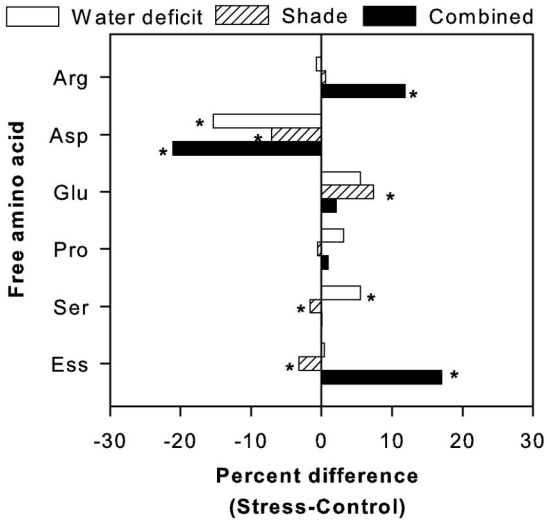

Fig. 1. Differences in percentage of total FAA accumulation (plants exposed to stress − control plants) of five FAAs that each comprised 10% or more of the total accumulation, and summed free essential amino acids, in plants that received 25% of full watering (W Deficit), 30% of full light (Shade), or both stress treatments (Combined). *Significant difference in FAA composition between stressed and control plants ($P < 0.05$, Tukey tests, n = 14–15 for water deficit stress, $n = 10$ for shade and combined stress). Arg, arginine; Asp, aspartate; Glu, glutamate; Pro, proline; Ser, serine; Ess, free essential amino acids.

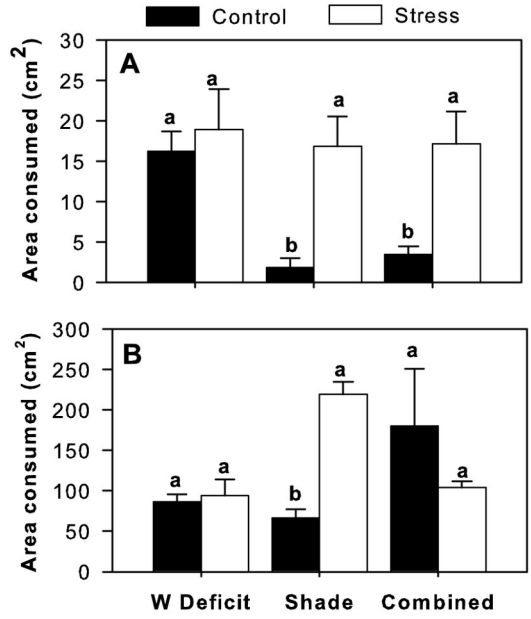

Fig. 2. Mean leaf area consumed ± SE by beet armyworms in (A) dual-choice and (B) no-choice tests on plants that received 25% of full watering (W Deficit), 30% of full light (Shade), or both stress treatments (Combined) compared with nonstressed controls. Bars within pairs with the same letter are not significantly different ($P > 0.05$, χ^2 tests for the dual-choice assay; Tukey tests for the no-choice assay; $n = 10$ plant pairs or plants, except $n = 7$ for the dual-choice assay for combined stress).

Combined stress increased proportional essential FAA content by 17% ($F = 20.5$, df = 1,18, $P < 0.001$; Fig. 1). Shade stress decreased essential FAAs by 37% (Table 3), and the essential proportion of total FAAs declined by 3.2% ($F = 9.34$, df = 1,18, $P = 0.007$; Fig. 1).

Beet Armyworm Feeding. Leaf areas consumed by beet armyworms did not differ between water deficit–stressed and control leaves in the dual-choice assay (Fig. 2A). Leaves from shade-stressed plants received 9.1-fold more damage ($\chi^2 = 6.9$, df = 1,10, $P = 0.009$) and leaves from combined stress plants received 4.9-fold more damage ($\chi^2 = 4.4$, df = 1,8, $P = 0.04$) than nonstressed controls. Water deficit and combined stress did not influence leaf area consumption in no-choice assays. Consumption was 3.3-fold higher on shade-stressed plants than on controls ($F = 66.8$, df 1,18, $P < 0.001$; Fig. 2B). The total leaf area available for feeding was reduced by 58% on combined stress plants (control, 614 ± 108 [SE] cm^2; combined stress, 260 ± 29 cm^2; $F = 9.94$, df = 1,18, $P < 0.01$). On a proportional basis, larvae consumed more leaf area on combined stress plants (42 ± 3.9%) than on controls (25.3 ± 4.9%; $F = 7.8$, df = 1,18, $P = 0.01$).

Beet Armyworm Survival. In whole-plant assays, larval survival on water deficit–stressed plants did not differ from survival on controls. Survival was reduced on shade-stressed plants (22 ± 6%) compared with their controls (95 ± 2%; $F = 78.2$, df = 1,18, $P < 0.001$). In detached-leaf assays, the survival of neonate larvae was 60% lower when reared on leaves from shaded plants than on leaves from control and water deficit–stressed plants (Table 4). Pupation was delayed by 2 d on shade-stressed leaves. Pupal length and weight were not affected by food source (Table 4).

Beet Armyworm Oviposition. Females laid 82% more eggs ($\chi^2 = 156.0$, $P < 0.001$) on nonstressed plants than on water deficit–stressed plants (Fig. 3). Conversely, 86 and 59% more eggs were deposited on shade ($\chi^2 = 238.2$, $P < 0.001$) and combined stress ($\chi^2 = 166.2$, $P < 0.001$) plants, respectively, than on controls (Fig. 3).

Discussion

Plant Phenology. Two weeks of water deficit, shade, or combined stress were sufficient to reduce plant growth and alter water-related measures on pigweed. The negative effects on plant height and weight are consistent with effects of water deficit on vegetable amaranth, *Amaranthus* spp., (Liu and Stutzel 2002) and cotton (Showler and Moran 2003) and the effects of shade on redroot pigweed, *Amaranthus retroflexus* L., (Begna et al. 2002, Rajcan et al. 2002). Increased stem elongation under shade occurs in other plants (Begna et al. 2002). Despite their elevated water potentials (Bray 1997, Garg et al. 2001, Showler 2002), osmotic adjustment, as in other *Amaranthus* spp. (Liu and Stutzel 2002), allowed water deficit–stressed

Table 4. Survival and time to pupation and pupal weight and length (mean ± SE) for groups of larvae fed leaves from plants grown under water deficit or shade stress

Variable[b]	Treatment[a]				
	Control	Water deficit	Shade	*F*	*P*
Larval survival (%)	95 ± 5.0a	90 ± 6.0a	35 ± 10b	14.0	<0.001
Time to pupation (days)	13.0 ± 0b	13.3 ± 0.12b	15.1 ± 1.09a	4.32	0.041
Pupal wt (mg)	78.7 ± 3.75a	72.5 ± 5.33a	77.4 ± 6.60a	0.43	0.663
Pupal length (mm)	11.3 ± 0.17a	11.0 ± 0.30a	11.3 ± 0.43a	0.43	0.661

Means in the same row followed by the same letter are not significantly different (Tukey's HSD, $P > 0.05$).
[a] Water deficit, 25% of full watering and full natural light; shade, full watering and 30% of full light; combined, 25% watering and 30% light.
[b] *F* and *P* values from ANOVA; $n = 5$ groups of larvae for survival test, df = 2,12; $n = 4$ (shaded) or 5 (water deficit and control) groups for pupation time and pupal size, df = 2,11.

A. palmeri plants to maintain water content. Shade stress reduced water potentials and increased water content (Henriksson et al. 2003). The effects of combined stress resembled those of water deficit alone (water potential) or shade alone (number of leaves, internode length, water content) and were not suggestive of synergism.

Nutritive Biochemical Components. The increase in protein content in water deficit–stressed pigweed is consistent with results in cotton (Showler and Moran 2003) and other plants (Chandra et al. 1998). Negative (Garg et al. 2001) or neutral (English-Loeb et al. 1997) effects on protein can also occur. Decreased photosynthesis (Smirnoff 1993), increased proteolysis (Ingram and Bartels 1996), and increases in drought tolerance proteins (Bray et al. 2000) can all influence protein levels. Lignification (thickening) and senescence (Smirnoff 1993) involve increases in peroxidase activities, and these processes accompanied leaf wilting under water deficit and possibly stem elongation under shade. Soluble carbohydrates responded positively to the two treatments involving water deficit stress in pigweed, as in many other plants (English-Loeb et al. 1997, Garg et al. 2001, Pinheiro et al. 2001, Streeter et al. 2001, Showler and Moran 2003). Decreases under shade could have been related to photosynthesis (Henriksson et al. 2003). Conflicts in plant signaling may have precluded changes in protein and peroxidase under combined stress, as suggested by the opposing water potential results for water deficit and shade alone.

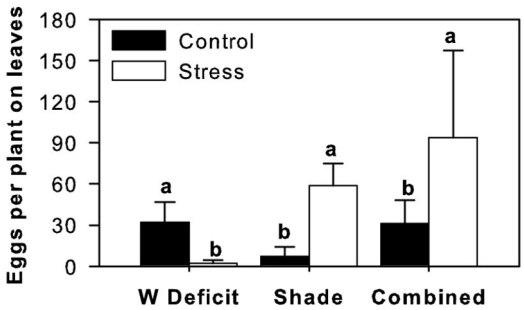

Fig. 3. Mean eggs laid by adult moths ± SE in dual-choice assays on plants that received 25% of full watering (W Deficit), 30% of full light (Shade), or both treatments (Combined) compared with nonstressed controls. Bars with the same letter are not significantly different ($P > 0.05$, χ^2 tests; $n = 10$ plant pairs).

The set of detectable FAAs in *A. palmeri* was similar to that of *A. hybridus* (Showler 2001) with the addition of alanine, tyrosine, and lysine. Water deficit and combined stresses positively influenced 10 and 12 individual FAAs, respectively, and these two similar FAA profiles broadly resembled the profile associated with water deficit in cotton (Showler 2002, Showler and Moran 2003). In both species, summed essential FAA and total FAA contents increased. The combined stress results showed that increases in FAAs were not dependent on increased soluble protein. Combined stress influenced arginine and summed essential FAA contents more strongly than did water deficit alone. Free proline accumulation was an indicator of water deficit stress (Kishor et al. 1995, Bray 1997, Showler 2002). Proline enhances water retention under deficit conditions (Bray 1997), acts as an osmoprotectant (Golan-Goldhirsh et al. 1989, Bray et al. 2000), and can ameliorate oxidative stress (Smirnoff 1993). With the exception of glutamate and aspartate, the effects of shade stress on FAA accumulations were largely neutral or opposite to those of the other treatments and included decreases in seven FAAs and summed essential FAAs. In cotton, aspartate decreased and arginine increased but no other changes in FAAs occurred (Showler 2002).

Beet Armyworm Feeding, Survival, and Oviposition. Given the assumed importance of nutrients in insect host selection and performance (White 1984, Mattson and Haack 1991, Bi et al. 1997, Showler 2002), the TAC and FAA results suggested that water deficit and combined stresses would have similar effects on beet armyworm feeding, survival, and oviposition. In contrast, the water content results suggested that the effects of shade and combined stress on beet armyworm would be most similar. The negative effects of all stress treatments on plant size and biomass could have modified the influences of nutrients and water in whole-plant bioassays.

Leaf area consumption by beet armyworm was not affected by water deficit. In contrast, feeding was elevated on drought-stressed cotton (Showler and Moran 2003). Consumption was elevated on pigweed plants exposed to shade or combined stress in the choice assay. Leaf beetles on cottonwood similarly prefer shaded leaves (Crone and Jones 1999). El-

evated water content and possibly other changes common to shade and combined stress were more important in influencing feeding than were nutrients (Waring and Cobb 1992) or antinutritive peroxidases (Bi et al. 1997). FAAs could be less important to feeding behavior in pigweed than in cotton because pigweed is nutritionally superior in FAA diversity and abundance (Showler 2001). In the no-choice assay on whole plants, combined stress may have led to a balance between water and nutrient stimuli, explaining why shade alone and not combined stress stimulated increased leaf area removal. However, reduced leaf areas increased the proportional damage inflicted by beet armyworms on combined stress plants compared with controls.

Despite being preferred by larvae, larval survival was reduced on leaves from shaded plants. Whole shaded plants had fewer leaves than controls, suggesting that enhanced leaf consumption and food depletion, combined with reduced tissue nutritive quality (TAC and essential FAAs), could have limited survival on shaded plants. Because pupal biomass was not affected, leaf area was likely not limiting for larvae on regularly replenished detached leaves. The increased mortality and prolonged development on detached leaves might have originated in neonate larvae if these larvae lacked the positive feeding response of older larvae to shaded tissues. Increased water content could have led to premature satiation in neonates feeding on tissues with reduced nutrient contents. Enhanced nutrients in water deficit–stressed plants did not benefit beet armyworm survival or pupal biomass, suggesting that nutrient levels were sufficient in nonstressed tissues. In cotton (Showler and Moran 2003) and tomato, *Lycopersicon esculentum* Mill (English-Loeb et al. 1997), survival was similarly not affected by water deficit, although the size or growth of larvae and pupae was reduced. Combined stress would be expected to enhance survival and pupal biomass because both nutrients and water content would be elevated, although food quantity could be limiting because both the number and area of leaves would be reduced.

Water deficit and shade altered the ability of females to select pigweed plants for oviposition. Noctuid moths can detect variation in TAC, FAAs, and other nutrients (Blaney and Simmonds 1988), but beet armyworm females did not respond positively to enhanced nutrients in pigweed. Tactile cues related to water deficit–induced wilting could have played a role in deterring oviposition. In contrast, females prefer water deficit–stressed cotton (Showler and Moran 2003). Increased water content could have stimulated greater oviposition on plants grown under shade and combined stresses. *Pieris rapae* L. females show a similar water-based oviposition preference (Wolfson 1980). Beet armyworms, as soft-bodied insects of tropical environments, may be highly sensitive to stimuli associated with water content (Showler and Moran 2003).

The changes in pigweed associated with the two treatments involving water deficit were consistent with trends in diverse plant species (Waring and Cobb 1992), and the shade treatment generated contrasting biochemical effects. Contrary to the plant stress hypothesis (White 1984, Mattson and Haack 1991), increases in protein and FAAs induced by water deficit in pigweed did not improve the performance of beet armyworm or increase feeding. Water content was a more consistent behavioral stimulant (Chapman 2003). Defensive changes, such as increases in trypsin inhibitors in *A. hypochondriacus* (Sánchez-Hernández et al. 2004) could have obscured the nutritive benefits of water deficit. The results show that relationships between larval feeding, larval performance, and adult oviposition are not always positive (Bernays and Chapman 1994, Berdegué et al. 1998). Water deficit–stressed plants suitable for larvae received reduced egg loads from females, whereas shaded pigweed plants preferred for feeding and oviposition were inferior for larval development, as was the case in water deficit-stressed cotton (Showler and Moran 2003). Pigweed is better than cotton as a survey host plant for beet armyworm because larvae and adults prefer it in the absence of stress (Sappington et al. 2001, Showler 2001, Carroll et al. 2003). None of the three stress treatments adversely affected leaf consumption by larvae. Shaded pigweed plants naturally intermixed within or bordering cotton plantings could detect early invasions by adult beet armyworm eggs and young larvae. Chronic water deficit should not negatively influence the use of pigweed as a survey host for larval damage. Abiotic stress influences the ways in which surveys for beet armyworm using pigweeds should be interpreted.

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